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# Contribution of $Na^+/H^+$ exchange to $Na^+$ overload in the ischemic hypertrophied hyperthyroid rat heart

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**Abstract**

**Objective:** The mechanisms responsible for intracellular ion homeostasis in ischemic hypertrophied myocardium are not fully known. Moderately hypertrophied hyperthyroid hearts (T3) are characterized by the bioenergetic changes and increased $Na^+/H^+$ exchange (NHE) activity comparable with those observed in humans and experimental models of hypertrophy. Here we test the hypothesis whether NHE inhibition in T3 heart improves ion homeostasis during ischemia and contractile function during recovery. **Methods:** We compared intracellular $H^+$ ($H_i^+$) and $Na^+$ ($Na_i^+$) accumulations during 28 min global ischemia in isolated perfused T3 and euthyroid (EUT) rat hearts with and without NHE inhibition by using $^{31}P$ and $^{23}Na$ NMR. Heart function was measured during control perfusion and 30 min following ischemic insult. **Results:** In T3 hearts ischemia caused: (1) faster and greater $Na_i^+$ accumulation (534±25% of preischemic level versus 316±22% in EUT, $P<0.001$); (2) lower acidification ($pH_i$ 6.66±0.66 versus 6.12±0.12 in EUT, $P<0.001$); and (3) faster hydrolysis of ATP. NHE inhibition (amiloride 1 mM) in T3 hearts lead to: (1) delayed and lower $Na_i^+$ accumulation by 35±5%; (2) faster and greater acidification ($pH_i$ 6.45±0.15, $P<0.05$); (3) delayed ATP degradation; and (4) improved heart function during recovery. When NHE was inhibited, all T3 hearts ($n=11$) recovered 68±10% of their preischemic rate pressure product (RPP), while only two untreated T3 hearts (from 11) recovered ~40% of preischemic RPP. **Conclusions:** These data suggest that NHE inhibition could be useful intervention for the prevention of ischemic/reperfusion cell injury and could improve the function of the hypertrophied heart after acute ischemia.



*Keywords:* Hypertrophy; Ion transport; Ischemia; Na/H-exchanger; Reperfusion

## 1. Introduction

Prospective studies have identified the presence of cardiac hypertrophy as the single most important indicator for high heart failure-related mortality [1]. The risk of acute myocardial infarction, developing life-threatening arrhythmias and sudden death increases six- to eightfold with the occurrence of myocardial hypertrophy [2,3]. Therefore, it becomes a pressing issue to identify potential targets for treatment of acute ischemia in the hypertrophied heart. One possible target is the $Na^+/H^+$ exchanger (NHE).

A critical characteristic of ischemia, which determines the extent of ischemic injury of the normal myocardium, is abnormal ion homeostasis. One metabolic consequence of ischemia is the rapid fall of intracellular pH ($pH_i$), from ~7.1 to values as low as $pH_i$ ~6.1 [4]. The fall of $pH_i$ activates the NHE [5,6]. While increased NHE activity leads to $H^+$ extrusion, it also moves substantial amounts of sodium ($Na_i^+$) into the cell [7–10]. Intracellular $Na^+$ overload contributes to ischemic and reperfusion injury [11]. The role of NHE inhibition in the preservation of the myocardium exposed to ischemic/reperfusion injury has

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been amply demonstrated for the normal heart [12,13]. Similar to results obtained from animal studies, a recent trial in humans demonstrated that inhibition of NHE in acute myocardial infarction, before reperfusion with precutaneous transluminal coronary angioplasty (PTCA), limited infarct size and improved left ventricular function after reperfusion [14].

Importantly, it has been recently demonstrated that cardiac sarcolemmal NHE activity (NHE-1) is increased in many different models of myocardial hypertrophy [15–18]. Furthermore, recent studies have presented convincing evidence that myocardial hypertrophy can be prevented by inhibition of NHE activity [18–20]. In addition, in vivo $^{31}P$ NMR spectroscopy studies have shown that the hypertrophied myocardium in man is characterized by lower phosphocreatine (PCr) to ATP ratio and higher inorganic phosphate (Pi) content, regardless of etiology and the degree of hypertrophy [21–23]. Taken together, these observations are important because ischemia in the hypertrophied myocardium may contribute to further deterioration of high-energy phosphate (HEP) stores that alter ion homeostasis and ultimately ventricular contractile dysfunction. The consequences of increased NHE-1 activity, the mechanisms responsible for maintaining intracellular ion homeostasis and energetics in hearts with lower energy stores, and how ion homeostasis changes in acute ischemia all remain unknown. The present investigation therefore, was undertaken to test whether NHE inhibition in the hypertrophied heart improves ion homeostasis and energetics during ischemia and contractile function during recovery.

The choice for experimental model is important. The moderately hypertrophied hyperthyroid (T3) rat heart has lower PCr/ATP and increased Pi content [24]. Because this bioenergetic profile mimics the profile now known to occur in human cardiac hypertrophy, we chose this model to study the regulation of $H_i^+$ and $Na_i^+$ in acute ischemia. We compared $H_i^+$ and $Na_i^+$ accumulations during ischemia in T3 and euthyroid (EUT) hearts with and without NHE inhibition. We used global ischemia (where the hearts cease beating) instead of low-flow ischemia (where the hearts continue to beat) so that the primary active ATPases are the $Na^+$ and $Ca^{2+}$ pumps, minimizing ATP consumption by myofibrillar ATPase. We used $^{23}Na$ NMR with shift reagents to measure changes in $Na_i^+$ content and $^{31}P$ NMR to measure $pH_i$ and calculate the cytosolic regulators of ion transport: ATP, Pi, and ADP contents.

## 2. Methods

### 2.1. Heart perfusion

The investigation conforms to the *Guide for the Care and Use of Laboratory Animals* published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996).

Male Sprague–Dawley rats were randomized into two age- and weight-matched groups (mean weight 380±10 g): 20 animals treated with thyroid hormone (T3) (3,3′,5-triiodo-L-thyronine, 200 μg/kg body weight, dissolved in 0.01 N NaOH, injected intraperitoneally once daily for 8 days) and 15 animals injected daily with vehicle (0.01 N NaOH) (EUT). Isolated hearts were perfused in the Langendorff mode at a constant temperature of 37 °C and constant perfusion pressure of 100 mmHg, as was described previously [4,24]. A water-filled balloon was inserted into the left ventricle (LV) through the mitral valve and connected to a pressure transducer (Statham P23Db Gould Instruments, Oxnard, CA, USA). Isovolumic contractile performance was estimated as the rate pressure product (RPP), the product of heart rate (HR) and LV developed pressure.

The standard perfusate was phosphate-free Krebs–Henseleit buffer (mM): NaCl 118, KCl 4.7, $MgSO_4$ 1.2, $CaCl_2$ 1.75, EDTA 0.5, glucose 11, $NaHCO_3$ 25 saturated with 95% $O_2$–5% $C0_2$, pH 7.4. For $^{23}Na$ NMR experiments one of two shift reagents was added to the buffer: 10 mM $Dy(TTHA)^{3-}$ (dysprosium triethylenetetraminehexaacetate) or 3.5 mM $Tm(DOTP)^{5-}$ (thulium 1,4,7,10-tetraazacyclododecane-*n*,*N*′,*N*″,*N*‴-tetramethylene phosphate obtained from Magnetic Resonance Solutions, Dallas, TX, USA). The ionic composition of shift reagent-containing buffers was adjusted to match standard buffer. Shift reagent did not affect cardiac function or HEP content.

### 2.2. Experimental protocols

Each heart underwent a stabilization period of ~20 min during which the magnet was shimmed and the heart function was stable. Then, one of two protocols was used. In the first protocol, hearts were perfused during the next 16 min for a baseline data defining cardiac performance, $pH_i$ and HEP content, then subjected to 28 min of global normothermic (37 °C) no-flow ischemia, followed by 30 min of reperfusion. In the second protocol, hearts were supplied either with amiloride (AMI; 1 mM) or ethylisopropylamiloride (EIPA; 10 μM) during 4 min, immediately before the onset of ischemia. NMR and cardiac performance were measured continuously throughout these protocols. Separate groups of hearts were used for $^{31}P$ NMR and $^{23}Na$ NMR experiments.

### 2.3. NMR measurements and calculations

NMR measurements were made using a GE-400 wide-bore Omega spectrometer (Fremont, CA, USA). $^{31}P$ NMR spectra were collected at a frequency of 161.94 MHz. The pulse angle used was 45° (23 μs pulse time) using a sweep width of ±3000 Hz and 2K data points. Partially saturated $^{31}P$ NMR spectra were obtained, averaging data from 104

free induction decays (total time 4 min) throughout each protocol. Spectra were analyzed using 20 Hz exponential multiplication and zero and first order phase corrections. Peak areas were corrected for saturation and estimated by using curve fitting program (NMR1, Syracuse, NY, USA) [4]. The area of the [β-P]ATP of spectra obtained under control perfusion was set to experimentally determined values of 10.3 mM and 10.7 mM in EUT and T3 hearts, respectively. These values were used as internal standards to calculate changes in the concentrations of ATP, ADP, PCr and Pi [4,24]. $pH_i$ was determined from the chemical shift of the inorganic phosphate resonance [4] The free energy level of ATP hydrolysis ($\Delta G_{\sim ATP}$) was calculated as: $\Delta G_{\sim ATP} = \Delta G^\circ + RT \ln[ATP][Pi]/ATP$ (kJ/mol) [25]. $\Delta G^\circ$ is standard free energy under physiological conditions and $R$, $T$ are the gas constant and the absolute temperature, respectively.

$^{23}$Na NMR spectra were collected at a frequency of 105.82 MHz. The pulse angle used was 90° (72 μs pulse time) using a sweep width of ±2000 Hz and 0.5K data points. Spectra were analyzed using 5 Hz exponential multiplication and zero and first order phase corrections. Peak areas were analyzed using NMR1 program.

### 2.4. Statistics

All presented values are mean±S.D. Statistical significance ($P<0.05$) of the results was determined by using repeated measures analysis of variance (ANOVA) followed by a Student's–Newman–Keuls test to analyze group comparisons.

## 3. Results

### 3.1. Characteristics of T3 rats and hearts

After T3 injections, body weights were similar (379±7 g T3 vs. 395±10 g EUT), but T3 hearts were larger (1.47±0.07 vs. 1.20±0.07 g). HR in T3 hearts was higher (349±25 vs. 282±24 beats/min, $P<0.05$), but there was no difference in RPP (29 500±2800 in T3 vs. 27 400±2600 mmHg in EUT). Coronary flows were not different between T3 and EUT (~16 ml/min per g wet weight).

Representative $^{31}$P NMR spectra from T3 and EUT rat hearts perfused under identical conditions show differences between the amounts of phosphate-containing compounds (Fig. 1). Compared to EUT hearts, [PCr] was 49% lower and [Pi] was 109% higher in T3 hearts; [ATP] and $pH_i$ were unchanged (Table 1).

### 3.2. HEP contents and $pH_i$ during ischemia

Ischemia caused a rapid depletion of [PCr] in both EUT and T3 hearts (Fig. 2). Although the rate of PCr utilization was faster in EUT hearts (~3.2 vs. ~2 mM/min), [PCr] in T3 hearts after 4 min of ischemia was only 5±4% of pre-ischemic values when in EUT hearts was 25±5% ($P<0.001$). Pi accumulation in T3 hearts in the first 8 min of ischemia was more than 2-fold higher than in EUT hearts ($P<0.05$) (Fig. 2) and remained significantly higher during the entire period of ischemia ($P<0.05$). [ATP] in EUT hearts slowly fell to non-detectable levels by 24 min (Fig. 2). In contrast, in T3 hearts [ATP] fell rapidly to

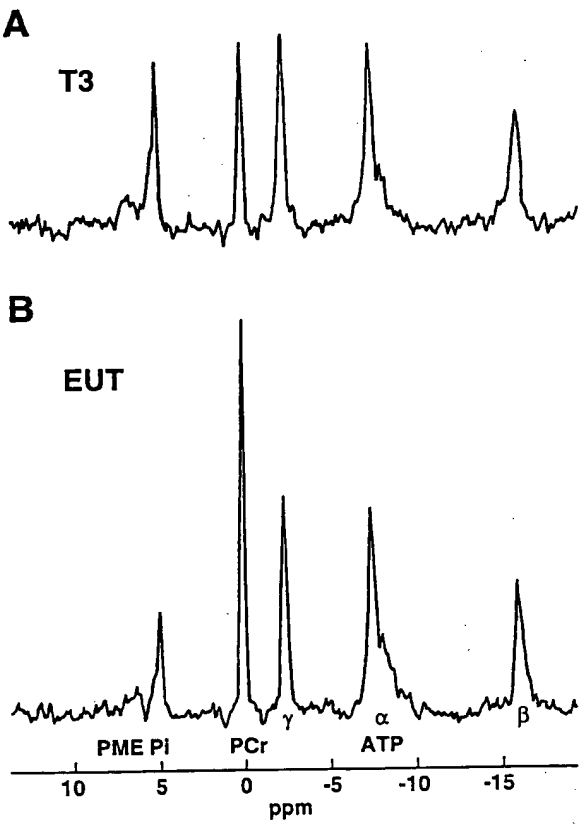


Fig. 1. Representative $^{31}$P NMR spectra obtained from an isolated Langendorff-perfused hyperthyroid (T3, A) and euthyroid (EUT, B) rat hearts during control oxygenated perfusion. Each spectrum represents 104 acquisitions obtained over a period of 4 min using pulse angle of 45° and recycle time of 2.18 s. Compared to EUT hearts, [PCr] was 49% lower and Pi was 109% higher in T3 hearts; [ATP] and $pH_i$ were unchanged.

Table 1
Metabolite concentrations and $pH_i$ in hearts from EUT and T3 rats

| | EUT | T3 |
|---|---|---|
| ATP (mM) | 10.6±0.6 | 10.5±0.4 |
| PCr (mM) | 17.0±2.1 | 8.6±1.3* |
| Pi (mM) | 4.5±1.1 | 9.4±1.2* |
| ADP (μM) | 54±9 | 116±9* |
| $pH_i$ | 7.10±0.02 | 7.14±0.02 |
| $\Delta G_{\sim ATP}$ (kJ/mol) | 59.2±0.2 | 55.4±0.2* |

All values are means±S.D.; * $P<0.05$ vs. EUT hearts.

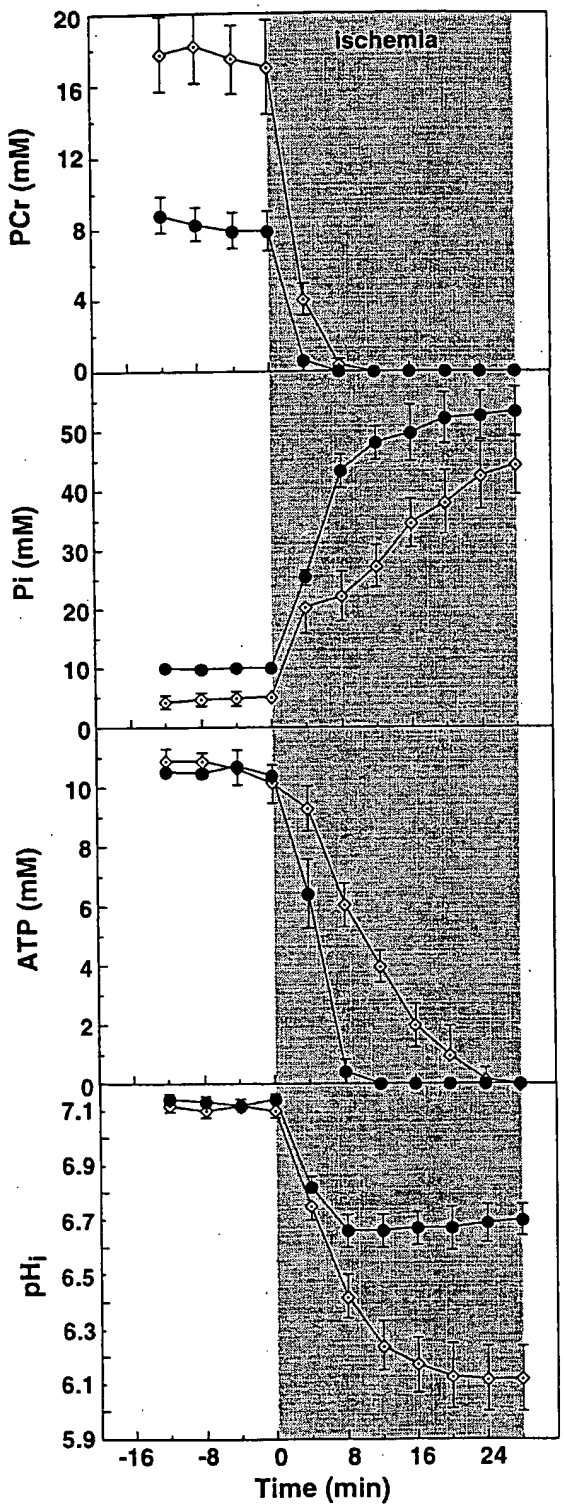


Fig. 2. Changes in PCr, Pi, ATP concentrations (mM) and in $pH_i$ before and during global no-flow ischemia. Values represent the mean±S.D. for hearts obtained from euthyroid (EUT, ◇ with dot, $n=7$) and treated with thyroid hormone rats (T3, ●, $n=11$). Statistical significance is stated in Section 3. Note the dramatic fall in PCr and ATP and rise in Pi in T3 hearts early in ischemia.

61±11% by 4 min ($P<0.05$, vs. EUT) and to 2±2% of pre-ischemic values by 8 min, when EUT hearts still contained 59±6% of their pre-ischemic [ATP] ($P<0.001$).

In the first 8 min of ischemia, $pH_i$ decreased linearly in EUT hearts (0.085 pH unit/min) and fell from 7.11±0.02 to 6.42±0.08 (Fig. 2). In T3 hearts, $pH_i$ fell at the same rate as EUT hearts only during the first 4 min (0.080 pH unit/min). Thereafter, $pH_i$ declined less rapidly and by 8 min fell to 6.66±0.06 ($P<0.01$, vs. EUT). Unlike EUT hearts, where $H_i^+$ continued to accumulate, there was no further $H_i^+$ accumulation in T3 hearts. At the end of ischemia $pH_i$ was 6.70±0.06 in T3 hearts compared to 6.12±0.12 in EUT hearts ($P<0.001$).

Compared to EUT hearts, T3 hearts are characterized by earlier PCr depletion, faster rate of Pi accumulation, markedly accelerated rate of ATP hydrolysis and higher $pH_i$ during ischemia.

### 3.3. $Na_i^+$ in EUT and T3 hearts during ischemia

Fig. 3 shows $^{23}Na$ NMR spectra obtained from EUT and T3 hearts perfused with both shifts reagents. Although spectral resolution with $Dy(TTHA)^{3-}$ is not as good as with $Tm(DOTP)^{5-}$, results obtained using the two shift reagents are indistinguishable. $Na_i^+$ areas for well-perfused T3 and EUT hearts were not different, 17.6±1.1 vs. 17.4±1.1 area units/g wet weight, respectively, whether measured by the NMR1 program, planimetry or 'cutting and weighing'.

In ischemic EUT hearts $Na_i^+$ increased slowly during the first 8 min, more rapidly between 8 and 16 min (3.8 times higher) followed by a slower increase (Fig. 4). By the end of 28 min of ischemia, $Na_i^+$ signal was 3-fold greater than pre-ischemia (316±22%). There was a markedly different pattern in ischemic T3 hearts. $Na_i^+$ began to increase within 2 min. The rate of $Na_i^+$ accumulation in the first 8 min was 6.3 times higher than in EUT hearts. The $Na_i^+$ signal continued to increase and by the end of ischemia was 534±25% of its pre-ischemic content. Thus, ischemic T3 myocardium accumulates more $Na_i^+$, more rapidly.

### 3.4. NHE inhibition

#### 3.4.1. $pH_i$ and $Na_i^+$

Perfusing EUT or T3 hearts with either AMI or EIPA 4 min before the onset of ischemia did not change either $pH_i$ or $Na_i^+$ content. Fig. 5 illustrates the different consequences of NHE inhibition in ischemic EUT and T3 hearts. NHE inhibition did not change $pH_i$ in EUT+AMI hearts (Fig. 5A). In contrast, NHE inhibition in T3 hearts led to a greater accumulation of $H^+$ (Fig. 5B). In the first 8 min of ischemia, the rate of fall of $pH_i$ was faster in T3+AMI hearts (0.074 pH units/min) than for untreated T3 hearts

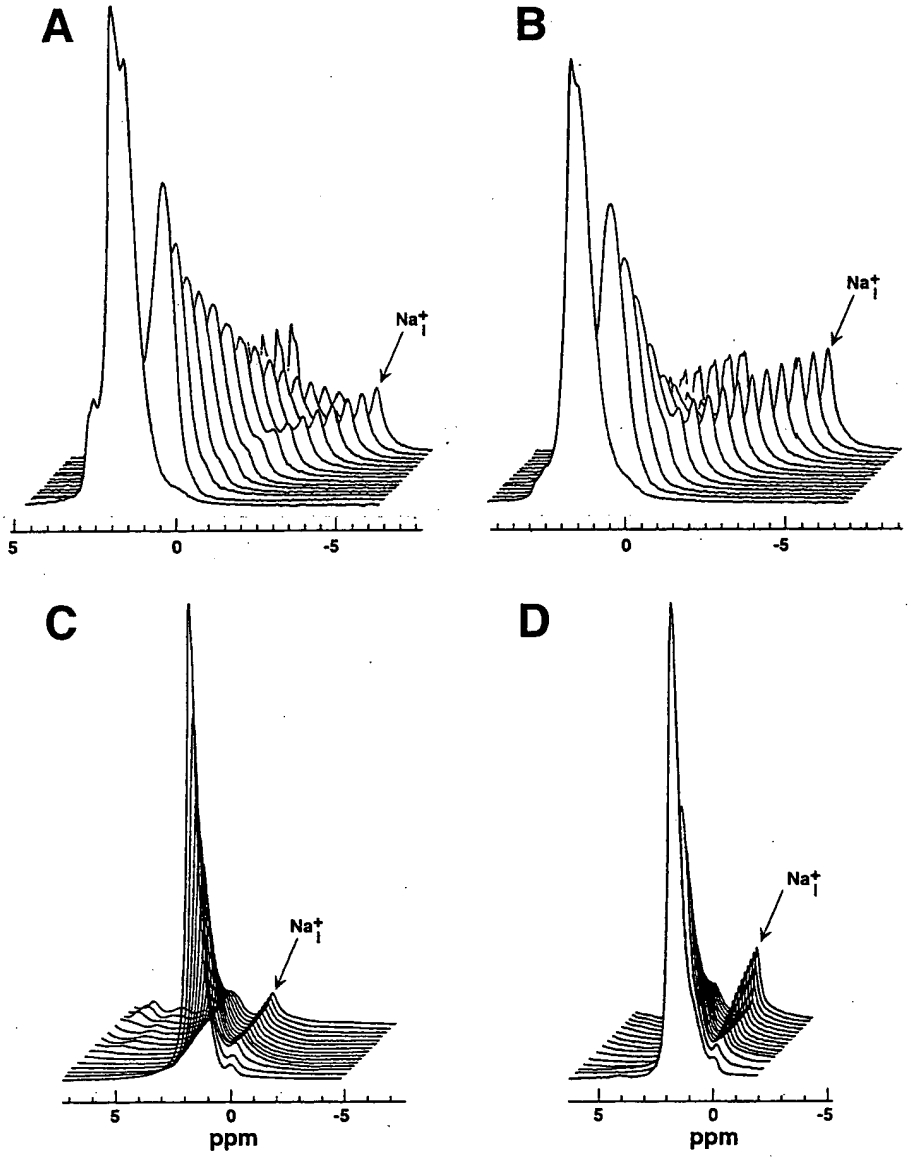


Fig. 3. Representative stacks of $^{23}Na$ NMR spectra obtained from EUT (A and C) and T3 (B and D) rat hearts subjected to 28 min of ischemia using $Dy(TTHA)^{3-}$ (A, B) and $Tm(DOTP)^{5}$, (C, D). The first spectrum of each set was obtained immediately before ischemia and the final spectrum was obtained at the end of ischemia; intervening spectra are at 2-min intervals. Note the different patterns of $Na_i^+$ increase in T3 and EUT hearts.

(0.060 pH units/min; $P<0.05$). After 12 min, $pH_i$ in T3+AMI hearts fell to 6.46±0.12 and remained below the pH observed in untreated T3 hearts (6.66±0.06; $P<0.01$) throughout the remaining period of ischemia.

NHE inhibition delayed and lowered $Na_i^+$ accumulation in both EUT and T3 ischemic hearts (Fig. 5). For EUT+AMI hearts, a significant increase in $Na_i^+$ content was observed only after 12 min (119±4%), when $Na_i^+$ content in EUT hearts was 196±8% of pre-ischemic values (Fig. 5A). $Na_i^+$ accumulation at the end of ischemia was 37±2% lower in EUT+AMI than in EUT hearts. NHE inhibition in T3 hearts greatly reduced the rise of $Na_i^+$ during first 6 min of ischemia (113±5% in T3+AMI versus 242±12% in T3). $Na_i^+$ increased linearly between 6 and 12 min and did not increase further. $Na_i^+$ accumulation by the end of ischemia was 35±5% lower in T3+AMI than in T3 hearts. To confirm that the changes in $Na_i^+$ accumulation were due to NHE inhibition, in three additional hearts we used EIPA, a more specific NHE inhibitor. The principal findings were the same (not shown). Thus, NHE inhibition leads to changes in both $Na_i^+$ and $H_i^+$ in T3 hearts and to the expected changes in $Na_i^+$ but not $H_i^+$ in EUT hearts.

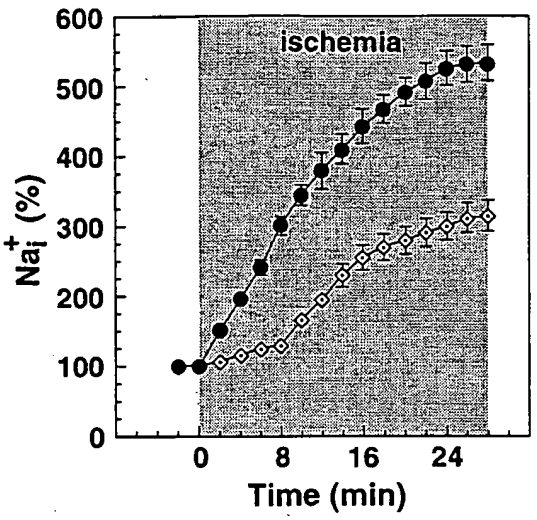


Fig. 4. Changes in % of preischemic level of $Na_i^+$ in 4 EUT (◇ with dot) and 5 T3 (●) rat hearts during 28-min ischemia. Values represent the mean±S.D.; during ischemia all values reached statistical significance ($P<0.01$ at 2 min; $P<0.001$ during the rest of ischemia, between 4 and 28 min).

#### 3.4.2. HEP content

NHE inhibition did not alter the changes in [PCr], [ATP] or [Pi] in ischemic EUT hearts (not shown). In contrast, ATP degradation was slower in T3+AMI than in T3 hearts (Fig. 6). In the 8th min of ischemia [ATP] in T3+AMI hearts was 32±17% of its pre-ischemic content, whereas untreated T3 hearts had only 2% ($P<0.001$). PCr hydrolysis was unchanged (Fig. 6) but the rate of Pi accumulation was slower (Fig. 6) ($P<0.05$). By 4 min of ischemia, NHE inhibition also attenuated the rise in [ADP] (447 μM in T3+AMI versus 1279 μM in T3) and the fall in $\Delta G_{\sim ATP}$ (~4 kJ/mol higher in T3+AMI hearts).

#### 3.4.3. Heart function during reperfusion

Recovery from ischemia was assessed in all hearts used for $^{31}P$ NMR experiments. NHE inhibition improved both the probability for recovery and the function of hearts that recovered, especially T3 hearts (Table 2).

## 4. Discussion

This study provides new insights into the mechanisms that lead to $Na^+$ and $H^+$ accumulation in the hypertrophied myocardium during acute ischemia. There are four main observations. First, the moderately hypertrophied myocardium from T3 rats exposed to acute ischemia accumulates more $Na^+$ and less $H^+$ compared with normal myocardium. Second, inhibition of NHE prior to ischemia leads to both lower $Na_i^+$ accumulation and higher $H_i^+$ accumulation. This is the first intact heart model to demonstrate concomitant effects on $H_i^+$ and $Na_i^+$ with NHE inhibition. Third, NHE activity is higher in hypertrophied T3 myocardium. Fourth, NHE inhibition in hypertrophied T3 myocardium improves recovery from ischemia, suggesting that NHE inhibition would be a useful addition to the armamentarium devised to protect the hypertrophied myocardium in acute ischemia.

Cardiac hypertrophy is the consequence of compensatory mechanisms by which the left ventricle adapts to increased pressure or volume overload, or to genetic defects. Mechanical stress triggers a growth response and results in numerous protein changes, including changes in the activity of sarcolemmal proteins [26]. Recent studies have provided evidence that increased NHE-1 activity is a primary factor mediating the anabolic state of cardiomyocytes during the development of many different models of hypertrophy [27]. However, no changes in the concentrations of $H_i^+$ have been detected in the hypertrophied myocardium under physiological conditions [27]. There are only limited data about changes in the accumulation of $H_i^+$ or $Na_i^+$ during acute ischemia in the hypertrophied myocardium [28,29].

### 4.1. NHE activity in the hypertrophied T3 heart

Moderately hypertrophied T3 hearts exhibited faster and greater $Na_i^+$ accumulation following the initiation of no-flow ischemia compared with normal euthyroid hearts. Greater $Na_i^+$ accumulation during ischemia has also been demonstrated in hypertrophied hearts caused by pressure overload exposed to low-flow ischemia [28,29]. Taken together, these results, using different stimuli to hypertrophy and different models of ischemia, suggest that a common property of the hypertrophied heart is greater $Na^+$ accumulation during an ischemic insult.

Many sarcolemmal currents participate in $Na^+$ entry into ischemic myocytes. The onset of ischemia induces the rise of $H^+$ and Pi and these stimulate NHE [5,6], $Na^+$/Pi cotransport [30] and $Na^+$–$K^+$–$2Cl^-$ cotransport [10]. Voltage-gated $Na^+$ channels are also activated, but their activities most likely cease after ~10 min of ischemia [31]. $Na^+/Ca^{2+}$ exchange [32], $N^+/HCO_3^-$ symport [33] and passive $Na^+$ influx also contribute. Because the Na pump is the only quantitatively important mechanism for $Na^+$ efflux, $Na_i^+$ overload occurs when $Na^+$ influx via all ionic currents exceeds the capacity of the Na pump to extrude $Na_i^+$ [34]. It has been shown that $T_3$ upregulates Na pump activity [34]. Here, during early ischemia in the T3 heart, [ATP] remained relatively high, [Pi] and $[H^+]$ were not different than for EUT hearts, and $\Delta G_{\sim ATP}$ for the $Na^+/K^+$-ATPase reaction was not limiting. Thus, $Na^+$ efflux should be similar or even higher in T3 hearts (due to higher Na pump activity) than in EUT hearts, leading to similar or lower $[Na^+]_i$. This is not, however, what we observed. Compared to EUT hearts, the rise of $[Na^+]_i$ in

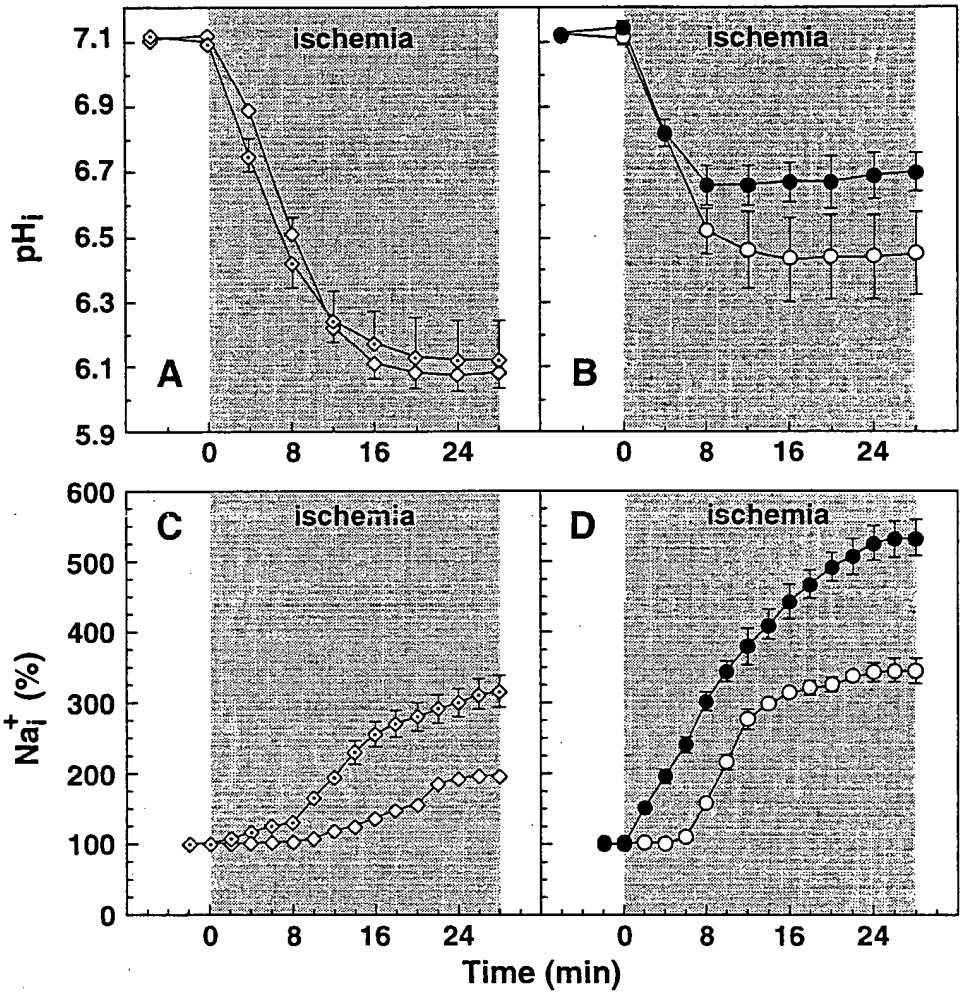


Fig. 5. Changes in $pH_i$ throughout ischemia in: seven EUT (◇ with dot) (A), 11 T3 (●) (B) rat hearts and supplied with 1 mM amiloride before ischemia: eight EUT + AMI (◇) (A) and 11 T3 + AMI (○) (B). Changes in $Na_i^+$ content throughout ischemia in: four EUT (◇ with dot) (A), five T3 (●) (B), four EUT + AMI (◇) (A) and six T3 + AMI (○) (B) hearts. Only T3 + AMI hearts demonstrated changes in both $Na_i^+$ and $H_i^+$; EUT hearts demonstrated changes only in $Na^+$.

ischemic T3 hearts was >6 times faster during early ischemia, and total $Na_i^+$ accumulation during ischemia doubled. In normal cardiomyocytes a substantial increase in ischemic $[Na^+]_i$ is caused by the $Na^+$ channel [35]. However, in the hyperthyroid myocardium, $Na^+$ uptake via $Na^+$ channels is decreased by ~50% [36]. Therefore, it is unlikely that the $Na^+$ channel would be the main route for $Na^+$ entry in the T3 heart. Thyroid hormone also reduces the $Na^+/Ca^{2+}$ exchanger activity [37] in cardiomyocytes.

An important carrier of $Na^+$ entry during ischemia is NHE. NHE activity has been shown to be upregulated by T3 in non-cardiac cells [38], but not in cardiomyocytes or in the intact heart. Increased NHE-mediated $Na^+$ uptake has been shown for papillary muscle from T3 rabbit [39]. The results presented here provide several lines of evidence showing that NHE activity is increased in the T3 heart.

First, in the present study, the impressive rise of $[Na^+]_i$ in T3 hearts parallels $H^+$ extrusion. An important and novel finding in our study was that pH did not fall by 1 log unit in T3 hearts as typically observed in EUT hearts made ischemic [4]: $[H^+]_i$ at the end of ischemic insult was 199 nM in T3 vs. 758 nM in EUT hearts. Thus, increased NHE activity in ischemic T3 hearts explains both faster and greater $Na_i^+$ accumulation and greater $H_i^+$ extrusion.

Second, in the first 4 min of ischemia, markedly greater ATP degradation, and hence higher $H^+$ production, occurred in T3 hearts compared to EUT hearts. This would be expected to result in lower $pH_i$. However, there was no greater acidification of T3 myocardium (6.82 vs. 6.75 for

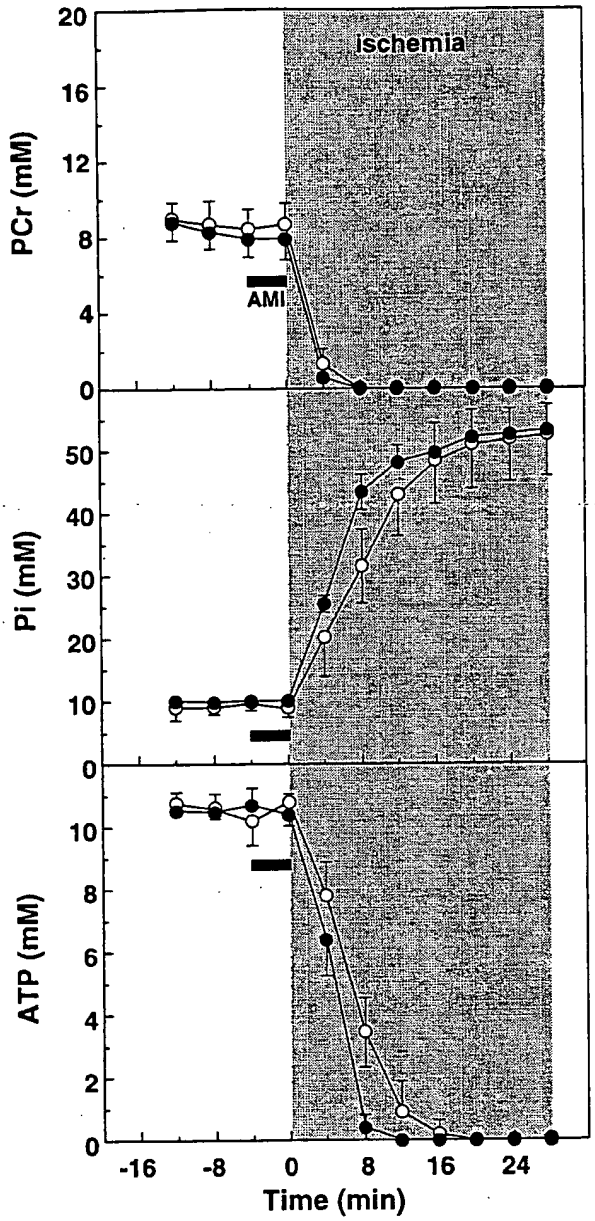


Fig. 6. Changes in PCr, Pi, and ATP concentrations (mM) before and during global no-flow ischemia in hearts from rats treated with thyroid hormone (T3, closed circles, $n=11$). Amiloride (1 mM) was infused during 4 min, immediately before the onset of ischemia (T3+AMI, open circles, $n=11$). The fall of [ATP] in T3+AMI hearts was delayed and ATP concentrations at 8 and 12 min of ischemic insult are significantly different from T3 hearts without amiloride ($P<0.05$).

T3 and EUT, respectively). This shows that T3 hearts were able to efficiently remove the additional $H_i^+$ load, providing further support for the conclusion that NHE activity is higher in T3 myocardium. Interestingly, Golden et al. [28] also observed significantly less acidification during

Table 2
RPP $\times 10^{-3}$ before ischemia and after 30 min of reperfusion (mean±S.D.)

| | Preischemia | | Reperfusion | | % Recovery |
|---|---|---|---|---|---|
| | *n* | RPP | *n* | RPP | |
| EUT | 7 | 28.7±2.9 | 3 | 13.4±3.2 | 47±10 |
| EUT+AMI | 8 | 26.1±2.3 | 7 | 16.2±5.4 | 65±15 |
| T3 | 11 | 29.6±3.3 | 2 | 15.1, 11.2* | 42, 43* |
| T3+AMI | 11 | 29.3±2.4 | 11 | 19.1±4.0 | 68±10 |

* Individual data for two recovered hearts.

low-flow ischemia in hypertrophied hearts from spontaneously hypertensive rats compared with hearts from WKY rats.

Third, although it is generally accepted that a major source for ischemic $H^+$ production is ATP hydrolysis, we cannot ignore other pathways of $H^+$ production in this no-flow ischemia model [40]. In experimental pressure overload hypertrophy caused by aortic banding, the rates of glycolysis from exogenous glucose and the rates of glycogenolysis are accelerated in hearts exposed to low-flow ischemia [41,42]. Importantly, it was shown that the rates of $H^+$ production during low flow ischemia were increased in these hypertrophied hearts [42], independently of coronary flow [43]. It is noteworthy that in the present study of no-flow ischemia, after all the ATP had been hydrolyzed in T3 hearts, we did not detect any further $H^+$ accumulation ($pH_i$ 6.66 vs. 6.70 at 8 and 28 min of ischemia), which suggests continued $H^+$ extrusion. In vitro experiments have shown that NHE is active at pH 6.8 but not below pH 6.6 [6,44]. The latter and the lack of any further $H^+$ accumulation after 8 min of ischemia in present study suggests sustained NHE activity during the entire period of ischemia in these T3 hypertrophied hearts reported here. Two different mechanisms of acid extrusion must be considered. First is the metabolic washout [45]. Based on studies using hypertrophied rat hearts caused by pressure overload, where ischemic lactate accumulation was no different than in control hearts [42], it is unlikely that this mechanism could explain the difference in ischemic $pH_i$ between EUT and T3 hearts reported here. Second, a recent study in rat cardiac cells clearly demonstrated that electogenic $N^+/HCO_3^-$ cotransporter plays an important role in acid extrusion [46]. There is no data that $N^+/HCO_3^-$ cotransport is different in EUT and T3 hearts.

If NHE activity is high in the ischemic T3 hearts, then NHE inhibition should change the pattern of $Na^+$ accumulation. Here, we have demonstrated that NHE inhibition caused a marked delay of the early rise of $[Na^+]_i$ and attenuated total $Na_i^+$ accumulation: at the end of ischemia, $Na_i^+$ was reduced by 189±21% in T3+AMI, compared with untreated T3 hearts. In the only other study with hypertrophied hearts, in that case caused by aortic-banding [29], inhibition of NHE during low-flow ischemia also

lowered $Na_i^+$ accumulation and prevented ischemic ventricular fibrillation.

NHE inhibition should also lead to higher $[H^+]_i$ (lower $pH_i$). Indeed, at the end of ischemia $[H^+]_i$ was 354 nM in T3+AMI hearts versus 199 nM in untreated T3 hearts ($P<0.05$). The study presented here is the only demonstration of both consequences of NHE inhibition during ischemia in the heart: a decrease in $[Na^+]_i$ simultaneous with an increase in $[H^+]_i$. Studies using EUT hearts have failed to observe this concordance [7,9,10, this study]. Here, we observed only a nonsignificant decrease in $pH_i$ euthyroid hearts when amiloride was present before ischemia (after 28 min of ischemia $[H^+]_i$ was 76 nM in EUT vs. 83 nM in EUT+AMI hearts). It is important to note that in EUT hearts, $pH_i$ was 6.75 and 6.42, 4 and 8 min after onset of ischemia, respectively. Clarke et al. [47] demonstrated that after 4 min from the onset of global no-flow ischemia $pH_i = pH_o$. It is highly likely that NHE should be inhibited by this $pH_o$ in euthyroid hearts. Therefore, for this reason, we (and others) have not been able to demonstrate the effect of NHE inhibition on $pH_i$ in EUT hearts.

The choice of inhibitor used here is important. The mitochondrial and the sarcolemmal membrane $Na^+/H^+$ antiporters represent distinct molecular entities. In the hypertrophied heart, acute ischemia leads to higher ATP utilization than in normal myocardium. Because a mitochondrial $Na^+/H^+$ antiport participates in ATP resynthesis, we wanted to reduce its possible contribution to the mitochondrial dysfunction. Amiloride was chosen specifically because, from all NHE inhibitors available, it does not inhibit mitochondrial NHE [48]. The specificity of AMI for the sarcolemmal NHE, coupled with the EIPA results presented here, indicates that sarcolemmal NHE plays a major role in the regulation of $[Na^+]_i$ and $[H^+]_i$ in hypertrophied myocardium during ischemia.

### 4.2. NHE inhibition and Na pump activity

Since ATP availability contributes to both $Na^+/K^+$-ATPase (Na pump) function [34,49] and NHE activity [50], the decreased HEP content in the hypertrophied myocardium determines not only the rate of ATP degradation during ischemia but also the extent of $H_i^+$ and $Na_i^+$ accumulation during ischemia. NHE inhibition leads to decreased $Na^+$ entry and thereby decreases the $Na^+$ load on the Na pump. In no-flow ischemia, where ATP hydrolysis by the $Na^+$ and $Ca^{2+}$ pumps exceeds ATP synthesis, the decrease in $Na^+$ load should cost less energy. Therefore, [ATP] should be higher, [ADP] and [Pi] lower and $\Delta G_{\sim ATP}$ higher. In this study, we observed that NHE inhibition led to this energetic profile in T3: NHE inhibition slowed the rate of ATP degradation. In this way, ATP availability plays a role in the preservation of the hypertrophic myocardium following ischemia. We suggest that this energetic mechanism couples NHE inhibition and Na pump activity.

### 4.3. Clinical implications and summary

In vivo studies of patients with varying degrees of cardiac hypertrophy have revealed changes in myocardial HEP content, independently of etiology [21–23]. Additionally, it is worth noting that similar alterations in HEP were detected in asymptomatic patients with moderate hypertrophic cardiomyopathy [51]. The latter suggests that energetic derangements are not only common to many forms of cardiac hypertrophy but also can be detected before myocardial dysfunction. Recently, several reports provide evidence that increased sarcolemmal NHE activity is an important factor in the development of different types of cardiac hypertrophy [27]. The T3 heart, an example of 'physiological' hypertrophy used in the present study, mimics these bioenergetic defects and increased NHE activity observed in experimental and human hypertrophied myocardium.

The present study shows that increased NHE activity in the hypertrophied T3 myocardium leads to increased $Na_i^+$ accumulation and to lower $[H^+]_i$ during ischemia. We also show that NHE inhibition not only decreased ischemic $Na_i^+$ accumulation but also increased $[H^+]_i$ during ischemia in these hearts, a unique demonstration of both consequences of NHE inhibition in the heart. Importantly, NHE inhibition markedly improved heart function during recovery from ischemia in hypertrophied hearts. Sarcolemmal NHE-1 inhibition leads to well described cardioprotective effects in ischemia/reperfusion of normal myocardium [11–13] and is the basis for a recent application for treatment of patients with acute myocardial infarction [14]. Our present results suggest several clinically important consequences of acute NHE inhibition for the ischemic hypertrophied myocardium. Decreasing $Na_i^+$ accumulation is cardioprotective in at least two ways: attenuating $Ca^{2+}$ overload and protecting HEP stores, which are needed to support $Na^+$ and $Ca^{2+}$ pump activities. This is especially important because cardiac hypertrophy, even in asymptomatic patients, is characterized by lower HEP content and a further loss of HEP during ischemia could lead to greater LV dysfunction. Secondly, decreasing $pH_i$ during ischemia can also be beneficial. Previously we found that decreasing $pH_i$ in ischemic heart has a salutary effect of inhibiting cytosolic 5′-nucleotidase, a key enzyme responsible for regulating the extent of ATP resynthesis during reperfusion [24]. Thus, both effects of NHE inhibition, namely decreased $Na^+$ and increased $H^+$ accumulation, are cardioprotective in the hypertrophied heart. Based on the results presented here, we suggest that NHE inhibition in the setting of acute ischemic injury in patients with cardiac hypertrophy due to essential hypertension, diabetes, valvular insufficiency and genetic

cardiomyopathy is an important area for future investigation.

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